"TO EVALUATE THE EFFECT OF LIGHT CURABLE FLUORIDE VARNISH ON ENAMEL DEMINERALIZATION DURING ORTHODONTIC TREATMENT"

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"TO EVALUATE THE EFFECT OF LIGHT CURABLE FLUORIDE VARNISH ON ENAMEL DEMINERALIZATION DURING ORTHODONTIC TREATMENT"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. T. SHOBBANA DEVI, M.D.S** Reader, Department of Orthodontics and craniofacial orthopaedics, Ragas Dental College and Hospital, Chennai.

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This is to certify that this dissertation titled **"TO EVALUATE THE EFFECT OF LIGHT CURABLE FLUORIDE VARNISH ON ENAMEL DEMINERALIZATION DURING ORTHODONTIC TREATMENT"** is a bonafide record work done by **Dr. Vidhu.S** under my guidance during her post graduate study period 2014-2017.

This dissertation is submitted to **THE TAMILNADU Dr.M.G. R MEDICAL UNIVERSITY,** in partial fulfillment for the degree **of MASTER OF DENTAL SURGERY in BRANCH V** -Orthodontics and Dentofacial Orthopedics. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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CONTENTS

Introduction

INTRODUCTION

Orthodontist strive to achieve an ideal occlusion and a beautiful smile for their patients that are functional, esthetic and stable. Despite technological advances and newer materials in the field of orthodontics, white spot lesions (WSLs) or enamel decalcifications around brackets remain a common complication in patients undergoing fixed appliance treatment. Carious lesions can be prevented by the proper use of rinses but depends on patient compliance

White spot lesion is defined as “subsurface enamel porosity from carious demineralization” that presents itself as “a milky white opacity, when located on smooth surfaces.” (81) and the white appearance is due to the changes in light scattering of the decalcified, porous enamel. These WSLs rarely progress into significant cavities. Orthodontic patients are more prone to develop WSLs than non-orthodontic patients, and these WSLs may present esthetic problems years after treatment.

Studies on the incidence of WSL in orthodontic patients (42) found that the incidence of at least one WSL in patients who underwent treatment with fixed orthodontic appliances was 50%; this compares to only 24% in an untreated control group. A recent study by Sandvik et al (78) confirmed that about 50% of the patients receiving orthodontic treatment developed one or more WSL during treatment, compared to 11% in an untreated control sample. Studies have shown that fixed orthodontic appliances induce a rapid increase in the amount of dental plaque and pH of the plaque is much lower than that in non-orthodontic subjects. [43,26]

WSLs can form within four weeks, which is typically within the time frame between subsequent orthodontic appointments. The presence of orthodontic attachments in the oral cavity make the mechanical removal of plaque somewhat difficult [68]. In addition to the difficulty in removing accumulated plaque, lack of compliance in maintaining adequate oral hygiene can predispose orthodontic patients to white spot lesions (WSL) [97]. To prevent decalcification and formation of WSL, a good oral hygiene protocol must be implemented, including proper tooth brushing with a fluoridated dentifrice. Fluoride toothpaste is the basis for all caries prevention. Fluoride concentrations below 0.1% should not be recommended for orthodontic patients [72]. For less compliant patients, the use of a fluoridated dentifrice alone is ineffective in preventing the development of carious lesions. Orthodontic patients are therefore requested to use a fluoride mouth rinse (0.05% NaF) daily in addition to fluoride toothpaste. Fluoride rinses have been shown to significantly reduce white spot lesions during orthodontic therapy. Unfortunately, these preventive measures depend on patient compliance [67].

Gontijo et al in his study [41] concluded that fluoride varnish could be considered an efficient preventive method to enhance enamel resistance against cariogenic challenges in less compliant patients during orthodontic therapy.

The advantage of fluoride varnish is that they are easy to use and do not depend on patient cooperation. Various in-vitro and in-vivo studies have been conducted in the past years explaining the role of conventional fluoride varnish. But what remains a question is the longevity and sustainability of these fluoride varnishes and whether repeated application at regular intervals is required [(3)]. Teeth that had fluoride varnish applied around orthodontic brackets showed a 35% reduction in demineralized lesion depth. The application of fluoride varnish is a preventive protocol. As fluoride varnishes have prolonged contact time with the tooth surface there is a significant amount of fluoride incorporation into enamel surface and further uptake compared to the other non-compliant methods.

Clinpro XT (3M ESPE, INDIA) is a highly filled, resin-modified glass ionomer-based light-curable fluoride varnish (RMGI-LCFV). At present, literature evidence on Clinpro XT is confined to reduced dentine hypersensitivity and its effect on preventing enamel demineralization is limited.

Various methods have been instituted to determine the extent of enamel demineralization caused by orthodontic treatment which include quantitative light immunofluorescent (QLF), photo micrographs of enamel surface, mineral analysis etc. In this study, we used scanning electron microscopy (SEM) and electron dispersive X-ray analysis (EDAX) for a qualitative as well as a quantitative analysis.

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. SEM allows the visualization of images at high magnification (50x -10.000 x and above) technique, an electron beam scans the surface of the sample to produce a variety of signals, the characteristics of these signals are depended upon factors like, including the energy of an electron beam and the nature of the sample, since a beam of electrons hit the sample and the response is collected by a detector, as described by Saghiri et al. [(89)] Sputtering of the dental sample is necessary as the teeth and other dental samples (composites, ceramics, cements, for instance) are conductive, with the usage of Au or Au-Pd target allows..

EDAX is a quantitative, and qualitative method for identification of chemical elements in a wide variety of samples. [(67)]

Therefore, the aim of our study was to evaluate the effect of a single application of light curable fluoride varnish (Clinpro XT) in preventing enamel demineralization adjacent to brackets during orthodontic treatment. The enamel surface was analyzed using scanning electron microscopy (SEM) and the mineral contents of enamel surface were analyzed using electron dispersive X ray analysis (EDAX).

Review of Literature

REVIEW OF LITERATURE

O'Reilly MM, Featherstone JD et al (1987) **[67]** their study reported that measurable demineralization may occur as early as one month after bracket placement.

Ogaard B et al (1988) [68] in his studies explain that the dissolution of the enamel's mineral structure creates an alteration of the refractive index when light shines upon it creating an opaque white appearance.

Chang HS, Walsh LJ, Freer TJ (1997) **[24]** et al in their study on enamel demineralization during orthodontic treatment described etiology and prevention states that White spot lesions (WSLs) are a precursor to decay. Their presence shows that the healthy balance between demineralization and remineralization on the enamel surface has shifted towards demineralization with subsequent loss of tooth structure. Like dental caries, WSLs may be considered a carbohydrate induced, bacterial infectious disease. Bacteria, in the presence of sugars, produce acids which lead to the demineralization of enamel structure.

Shungin D, Olsson AI, Persson M et al (2010) [80] did a study of orthodontic treatment-related white spot lesions: a 14-year prospective quantitative follow-up, including bonding material assessment and found that the generated WSL may be remineralised back to normal, remain stable or progress to a cavitation depending on the oral environment.

Tufekci E et al (2011) [90] reported that even if a WSL does not progress to cavitation requiring restoration, it can leave a permanent unaesthetic blemish on the tooth's surface which is a chalky white or brown lesion. WSLs are a common complication of orthodontic treatment and are of concern because the enamel on the buccal surface of teeth would otherwise have low susceptibility to caries

Blake J. Maxfield,a Ahmad M. Hamdan et al (2012) [18] in their study, which aimed to assess the perceptions and level of awareness of patients, parents, orthodontists, and general dentists toward the development of white spot lesions during orthodontic treatment concluded that, all groups indicated that white spot lesions was not attractive from the overall appearance of a finished orthodontic patient, attributed primary responsibility for the prevention of white spot lesions to the patients themselves, and indicated that the general dentist should be responsible for treating white spot lesions. Most parents were concerned that their child would develop white spot lesions during treatment; however, most indicated that they would still recommend braces even if their child had white spot lesions after treatment. Most patients and parents were aware that white spot lesions were caused by inadequate or improper brushing and flossing during orthodontic treatment.

Khalaf K. Et al (2014) [48] reported that orthodontists and patients come to notice white spot lesions after removal of the fixed appliances, especially since the lesions often form in the maxillary anterior teeth.

Gorelick et al. (1982) [42] looked at the distribution of lesions throughout the dentition. According to him the highest incidence of white-spot lesions was observed in the labio-gingival area of the maxillary lateral incisors. The segments with the highest incidence of lesions were the maxillary anterior and mandibular posterior teeth, while the maxillary posterior and mandibular anterior had a lower incidence of new lesions. The authors argued that this may have been related to the rate of salivary flow in the areas, with an increased flow allowing for remineralization.

Artun J, Thylstrup A. Et al (1989) [7] in their study of surface changes of carious enamel lesions after inactivation found that the removal of the orthodontic appliance has been shown to allow for an arrest of the cariogenic lesion and an improved appearance over time. However, white spot lesions often persist without more aggressive treatment, such as full restorative dentistry.

Mattousch TJ, van der Veen MH, Zentner A. Et al (2007) [59] followed up on 51 subjects for 2 years after treatment and reported an overall improvement in the first six months after debonding, but no further improvement durig the next two years.

Chapman JA, Roberts WE, et al (2010) [25] reported that the incidence of white spot lesions in orthodontic populations have been reported at 23-72.9%. Treatment duration, poor oral hygiene scores, sugary food consumption, earlier age at the start of treatment and topical fluoride use have been correlated with incidence of lesions.

A systematic review by **Marco Migliorati, Luisa Isaia, et al (2015)** **[54]** stated that an optimal oral health maintenance during orthodontic treatment should be a gold standard in order to prevent white spot lesions.

A practice-based evaluation of the prevalence and predisposing etiology of white spot lesions is a study done by **Matthew D. Brown; Phillip M. Campbell et al (2016)** **[58]** which concluded that Approximately 28% of orthodontic patients in private practice settings develop WSLs.

Geiger et al. (1988) **[39]** have reported that the incidence of a WSL does not correlate positively with age; however, there are more advanced cavitated lesions in those less than 13 years of age.

Tufekci et al (2011) [90] found that males develop more white spot lesions than females. Additionally, males tend to have a greater severity of demineralization than females once the disease develops However, it is unlikely that a true gender-based risk exists. The apparent gender influences may be due to compliance with hygiene and preventive measures.

Al Maaitah EF, Geiger AM, Gorelick L, Mizrahi E et al (2011) **[1]** There is no clear sexual predisposition to WSLs with both males and females being equally at risk for disease development.

Artun and Brobakkan et al (1986) **[6]** also focused on tooth surfaces affected by decalcification. The investigators looked at 120 orthodontic patients treated in two different private practices. They found that the majority of buccal surface lesions were located in the gingival areas of the crowns of maxillary lateral incisors, mandibular canines, and mandibular premolars.

A study by **Julien et al. (2013)** [47] used digital photographs of 885 patients before and after treatment and found that the maxillary laterals and canines were the most affected teeth, with a greater incidence on the left maxillary lateral incisor when compared to the right.

More recently, **Khalfa et al. (2014)** [48] examined intraoral photographs of 45 patients and reported the highest incidence of white spot lesions were in the gingival areas of maxillary lateral incisors and canines, maxillary and mandibular premolars and first molars.

Wiechmann D, Klang E Et al (2015) [94] in their study found that in contrast to labially bonded orthodontic brackets, the use of a lingual appliance has been shown to have a significantly lower incidence of white spot lesions. Tooth surfaces bonded with a lingual retainer for a prolonged period of time are also reported to have no enamel decalcifications.

Michael Knösel, Elisabeth Klang et al (2016) [60] did a study on Occurrence and severity of enamel decalcification adjacent to bracket bases and sub-bracket lesions during orthodontic treatment with two different lingual appliances and stated that the use of lingual appliances instead of conventional labial appliances is advocated, in order to achieve a reduction in frequency of enamel decalcifications in multi bracket patients.

Benson et al. (1998) [14] evaluated three methods of enamel demineralization assessment: direct visual, microscopic and photographs. The authors concluded that the assessment of enamel demineralization from photographs was more reproducible than direct clinical observation utilizing only the naked-eye.

Ekstrand KR, Martignon S, Ricketts DJ, Qvist V et al (2007) [31] stated in their study that combined knowledge from a visual assessment, the location of a lesion and tactile sensation have been shown to be a valid means of detecting enamel decalcification.

According to **Chapman JA, Roberts WE, et al (2010)** [25] colour photography has also been found to be a powerful method of recording the presence of early enamel demineralization. Comparing image analysis of digital photographs with direct clinical examination showed excellent agreement.

Eissa, et al (2013) [66] found that quantitative light-induced fluorescence as well as computerized image analysis have been reproducibly used for the measurement of white spot lesions in many studies. Many of these methods require special equipment for the quantification of the demineralization area.

Boye U, Willasey A, et al (2013) [19] in their study of " comparison of an intra-oral photographic caries assessment with an established visual caries assessment method for use in dental epidemiological studies of children " concluded that in the absence of sophisticated image analysis tools, visual inspection of routinely taken intra-oral photographs has been shown to be equally effective. Comparing the gold standard method of clinical examination with an assessment of intra-oral photographs show equivalent diagnostic utility.

A study by **Almosa et al. (2014)** [2] compared the use of photographs to visual dental examination. Images were taken perpendicular to the facial surface of anterior and premolar teeth and subsequently visual examination was done. The study showed good agreement between clinical examination and visual inspection of digital photographs.

Cara C. Miller; Girvan Burnsideb (2016) [21] in their study stated that quantitative light induced fluorescence as an effective technique to assess the plaque accumulation and enamel demineralization in orthodontics

Ogaard B, et al (1988) [68] reported that a high standard of oral hygiene is required to prevent WSLs during orthodontic treatment. Ideal orthodontic patients should have a low caries risk and excellent hygiene habits before the start of treatment. Patients are often asked to brush a minimum of three times a day with a fluoridated dentifrice.

According to **Geiger AM, Gorelik L et al (1988)** [39] Professional dental scaling and oral hygiene instructions at regular intervals have been found effective in reducing the incidence of WSLs. In addition, this study has found a negative association between compliance of preventive strategies at home and the incidence of WSLs.

According **Sudjalim TR, Woods MG, et al (2006) [(85)]** Management involves preventing demineralization during orthodontic treatment as well as remineralising lesions once they have occurred. Generally, prevention is better than cure with any disease process and this holds true in the management of WSLs. Many preventive strategies are under investigation. From the literature, available at present, it is apparent that the exact combination of strategies required to prevent the occurrence of WSLs is still unknown.

Noble J, Costa MR, et al (2009) [(65)] said that brushing and flossing technique should be demonstrated to the patients. The use of an electric toothbrush can be recommended as it leads to a lower amount of plaque than manual tooth brushing with fixed appliances in place. They also reported that the diet that is high in fermentable carbohydrates presents an increased risk of decay. Patients are advised to reduce their intake of complex carbohydrates, carbonated drinks.

B. Ogaard et al (1990) [(70)] stated that fluoride is known to increase the rate of remineralization, although complete in vivo repair may be inhibited by precipitation of fluoride in the enamel surface. In vivo remineralization is initially very fast, especially for surface-softened lesions. For longer periods, the remineralization rate is extremely slow. A fluoride solution at low pH has been found to be more effective in caries model studies than neutral fluoride agents which might be due to the formation of a larger depot of calcium fluoride.

Jacob M. Ten Cate et al (1999) [(99)] in their study of current concepts of the theories of the mechanism of action of fluoride stated that The caries-preventive effect of fluoride is mainly attributed to the effects on demineralization/remineralization at the tooth-oral fluids interface Low fluoride levels are found in saliva after tooth brushing with fluoride containing dentifrices. Similar concentrations are ineffective in interfering with the processes of growth and metabolism of bacteria, and also do not result in a significantly reduced dissolution of tooth mineral as a result of (firmly bound) fluoride incorporation. Fluoride slow-release devices, in the form of fluoride-releasing restorative materials, may serve to increase the fluoride levels in saliva and plaque to levels at which caries can be prevented, also in high-risk patients.

Warren DP, Henson HA, Chan JT. Et al (2000) [93] also documented the disadvantages of using fluoride varnish in their study on dental hygienist and patient comparisons of fluoride varnishes to fluoride gels. They said that the major disadvantages are temporary discoloration and increased appointment time per patient. Reapplication at regular intervals is necessary due to removal of the varnish by mechanical brushing. Its use may be necessary in those high-risk patients unable to carry out proper oral hygiene. Though the majority of patients find the presence of varnish on their teeth acceptable, some patients dislike its presence as a thin film on their teeth or they find the taste of the varnish objectionable. Also, some orthodontists are reluctant to use fluoride varnish due to the increased chair time required for varnish application.

In a study by **Ogaard B, (2001)** [69] it was found that topical fluoride application causes the formation of a calcium fluoride-like material on the enamel surface which acts as a fluoride reservoir and is present some weeks after orthodontic treatment starts.

Arends J, Lodding A, Petersson LG, Seppa L. Et al (1980) [(4)] in their studies found that the benefit of fluoride varnish use includes longer contact time with enamel due to enhanced adherence to tooth structure resulting in increased length of time available to incorporate the fluoride into the enamel surface. This longer exposure period to fluoride increases the amount of fluoride retained in enamel, therefore enhancing the formation of fluoridated hydroxyapatite, and reduces the acid solubility of enamel. Fluoride varnish may be necessary in patients with strong gag reflexes, where delivery of fluoride in trays is difficult. An additional benefit of fluoride varnish is that a prophylaxis removal of plaque is not required before application and the varnish is not inactivated by dental plaque.

An initial Cochrane review by **Benson PE ,ParkinN et al (2004)** [(13)] on fluorides for the prevention of WSLs, has found that the exact treatment prescription that causes a reduction in WSLs in orthodontic patients is still debatable due to insufficient research available during the time of review.

A review by **Chadwick BL, Roy J et al (2005)** [(23)] The use of topical fluorides in addition to fluoride toothpaste appears to reduce the incidence of decalcification in patients undergoing orthodontic treatment with fixed appliances. Decreased incidence of decalcification is found in populations with both fluoridated and nonfluorinated water supplies. Several preparations have been shown to reduce the incidence of decalcification in patients undergoing orthodontic treatment with fixed appliances, but none appears to be superior Various formats (mouth rinses, gels, high-potency gels, varnishes) have been shown to reduce the incidence of decalcification in patients undergoing orthodontic treatment with fixed appliances, but none appears to be superior. High-potency preparations appear to offer benefits. It is not possible to recommend which topical preparations or schedules provide the greatest decrease in decalcification

A systematic review by **Benson PE, Shah AA et al (2005)** **[15]** has recommended the daily use of 0.05% sodium fluoride mouthwash during treatment. However, this recommendation is based on research carried out on non-orthodontic patients of similar age groups.

Disadvantages of a varnish were summarized by **Bishara SE, Ostby AW (2008)** **[17]** in their review which include temporary discoloration, limits in frequency of application and increased costs/chair time. The study also stated that preventive fluoride therapy in addition to fluoridated dentifrice usage is imperative in contemporary orthodontic practices, and has proven to inhibit caries formation and decrease enamel demineralization. The presence of fluoride at the time of acidic attack may considerably slower the rates of demineralization. During both periods of demineralization and remineralization, enamel lesions preferentially absorb fluoride ions onto their partially demineralized hydroxyapatite crystals or redeposit fluoride as fluoridated hydroxyapatite. Fluoride's ability to prevent and arrest the caries process is dependent upon three mechanisms of action: 1) inhibiting demineralization when fluoride is present on the crystal surfaces during an acidic challenge, 2) inhibiting bacterial metabolism after diffusing into the bacteria as hydrogen fluoride when plaque is acidified, and 3) enhancing remineralization and forming a low-solubility fluoridated hydroxyapatite. Not only can fluoride decrease demineralization, it can also remineralize existing early white spot lesions. They also explain in their study on White spot lesion: Formation, Prevention and treatment about Fluoride as widely accepted to have a key beneficial role in the prevention of decay in children. When fluoride is incorporated into the enamel, fluorapatite crystals are formed which have an increased resistance to acid attack compared with normal hydroxyapatite crystals. During orthodontic treatment fluoride application, may be topical (mouthwashes, varnishes, gels, toothpastes etc.) Or incorporated into orthodontic materials (cements, modules, adhesives etc.). Various preparations of fluoride, such as stannous or sodium fluoride are also available.

Gontijo L et al (2007) [(41)] in their studies stated that fluoride varnish provides a protective coat over the tooth, adheres longer to the tooth’s surface and has a higher concentration of fluoride compared with fluoride rinses. A varnish may also be placed in dental areas at higher risk of demineralization.

Another Cochrane review by **Marinho VC (2009)** **[55]** on fluoride use for caries in general has further emphasized that more research using better methodology is required before an exact modality and strength of fluoride can be recommended for orthodontic use.

Various authors like **Leizer C (2010)** **[51]** reported that the ideal concentrations of fluoride should be considered. High levels of fluoride used to treat existing white spot lesions will only remineralize the surface layer of the lesion. This superficial layer might prevent calcium and phosphate from penetrating to the deeper layers of the enamel, thus inhibiting deeper remineralization and limiting the cosmetic improvement of the WSLs. Thus, low fluoride levels should be used in order to promote remineralization of the entire depth of the lesion. The goal in the oral hygiene promotion should be frequent low concentration fluoride exposure to prevent demineralization and to completely repair existing lesions.

Zipkin Levine, Wu, Reynolds et al (1970) **[98]** stated that fluoride has several mechanisms of action to aid in the prevention of dental decay. Fluoride improves saliva remineralization effects when it combines with hydroxyapatite to form fluorapatite, resulting in less soluble and more resistant enamel when faced with an acidic challenge. Also, the fluoride in plaque interferes with bacterial metabolism of carbohydrates. Fluoride is effective in inhibiting enolase, an enzyme necessary for fermentation, because enolase is sensitive to fluoride even at low levels. Subsequently, oral bacteria have a decreased-acid production. Additionally, it can be effective in inhibiting bacterial colonization on the tooth surface via competitive binding. Due to its electronegative properties, fluoride competes with bacteria for these binding sites and prevents adhesion. However, the main mechanism of action of fluoride is the remineralization of affected enamel. After an acid attack, fluoride ions react with calcium and phosphate of hydroxyapatite to form fluorapatite and can be incorporated into the partially damaged enamel structure, resulting in the remineralization of the surface. For every two fluoride ions, ten calcium ions and six phosphate ions are required to form one unit cell of fluorapatite [Ca10 (PO4) 6F2]

Biestrock et al.. Harris et al. (1998) **[11]** This incorporation of ions into the lost mineral of caries lesions will slow down, and may even stop, the progression of the lesion actually concludes that the resulting enamel is more resistant to subsequent demineralization than the original one. While persistent, low levels of topical fluoride exhibit many beneficial properties, excessive amounts may demonstrate adverse effects.

García-Godoy et al., Øgaard, and Zero et al (2008) **[37]** in their studies has indicated that increased levels of topical fluoride will lead to rapid mineral precipitation on the enamel surface and subsequent obliteration of surface enamel pores. Therefore, high doses of fluoride used on porous white spot lesions affect mostly the outer surface, thereby inhibiting complete remineralization of the lesion in the subsurface areas a hard enamel shell is created with the remaining arrested demineralized lesion . For this reason, it is not recommended to apply high concentrations of fluoride (such as fluoride varnish) to areas of decalcification since the outer enamel shell will be sealed, thus preventing any further remineralization from occurring. Essentially, this will allow the white spot lesion to remain indefinitely. Higher amounts of topical fluoride also have the potential to be ingested, increasing the amount of systemic fluoride intake. In younger patients (under the age of six), this may lead to an increased incidence of dental fluorosis or potentially fluoride toxicity

Anurag Mehta, Ganesh Paramshivam et al (2015) [(3)] in their study evaluated the effect of a single application of Clinpro XT (3M ESPE, Pymble, New South Wales, Australia), a light-curable fluoride varnish, on enamel demineralization adjacent to orthodontic brackets. They concluded that Clinpro XT light-curable fluoride varnish may be a reasonable alternative in the reduction of enamel demineralization around orthodontic brackets, especially in noncompliant and high-risk patients. A single application of Clinpro XT, an LCFV, can prevent demineralization in long-term clinical situations up to 120 days and may be a useful alternative in noncompliant and high-risk patients.

Federico Perrini, a Luca Lombardo, et al (2016) [(36)] in their study of Caries prevention during orthodontic treatment: In-vivo assessment of high-fluoride varnish to prevent white spot lesions concluded that Periodic application of fluoride varnish tends to protect against white spot onset, but not significantly so in patients with excellent oral hygiene. Fluoride varnishes, together with other prevention strategies, may be a useful aid to protect the enamel in noncompliant patients (those with a disability or poor hygiene education.

According to **Wiltshire WA et al (1996)** [95] in their study states that similar to the adhesives, fluoride releasing elastomerics have not seen popular use in orthodontics. This is likely due to their rapidly declining fluoride release after initial placement and their ineffective force levels compared with regular elastomerics. **Wiltshire WA** also stated that elastomerics can enlarge when placed in the mouth, which increases their surface area to which plaque can attach.

Hong Chen, a Xingguang Liu, et al (2013) [44] in their systematic review on the effect of remineralizing agents of white spot lesions after orthodontic treatment concluded that systematic review indicated a lack of reliable evidence to support the effectiveness of remineralizing agents for the treatment of post orthodontic WSLs.

Benson PE, et al (2005) [15] in his study concluded that fluoride releasing materials have the advantage of not being dependent on compliance, but their disadvantage is that many of them release large amounts of fluoride initially but the levels drop down to sub-therapeutic levels throughout orthodontic treatment. Glass ionomer cement (GIC) and resin modified glass ionomer cement (RMGIC) can prevent demineralisation around orthodontic brackets when compared to resin adhesives during treatment.

A systematic review of **Rogers S, Chadwick B Treasure E (2010)** **(76)** concerning the question of whether GIC as an orthodontic adhesive result in less demineralization compared to traditional resin adhesives concludes that evidence is very weak in favor of GIC adhesives being protective. This study also mentions that GIC adhesives have a higher rate of debonding which may contraindicate their routine use in orthodontics.

Behnan SM, et al (2010) **(10)** in his study ,he documented about sealers Similar to those sealers that prevent caries in molars by being placed over fissures, resin sealers can be placed over the buccal surface of teeth with orthodontic brackets. This forms a physical barrier against both acid attacks and certain oral bacteria. The main problem with resin sealers is their low wear resistance causing them to be abraded by toothbrushes and requiring reapplication. The advantages include that their preparation may include fluoride or antimicrobial components and that they are not reliant on patient compliance. One in vitro study has found resin sealants and fluoride varnish to be superior in protecting against WSLs compared to CPP-ACP products applied topically and incorporated within cements. The authors of this study warn that evidence from randomized clinical trials is required before firm conclusions can be made. Another published study has found no significant difference using a fluoride releasing sealant compared to traditional resin adhesives.

A novel RMGIC was developed by **Xiaoying Wang, Bianhong Wang, and Yanhua Wang et al (2015)** **(92)** with NAg for the prevention of white spot lesions. The results showed that RMGIC containing NAg had much stronger antibacterial effects than did the commercial RMGIC control, and the incorporation of NAg into RMGIC could combat white spot lesions both beneath and away from the orthodontic brackets. These benefits were achieved without compromising the enamel shear bond strength and do not require patient compliance. Hence, the NAg-containing RMGIC might be more effective in preventing white spot lesions.

Danna Mota Moreiraa; James Oeib et al (2015) **[28]** developed a novel antimicrobial orthodontic band cement with in situ–generated AgNPs for the prevention of white spot lesions. These resins have comparable mechanical properties of controls, controlled and sustained Ag+ ion release, significant bacterial inhibition in vitro, and excellent biocompatibility

Sotiria Gizani, Georgia Petsi, Svante Twetman (2015) **[82]** **et al** did a study on the effect of the probiotic bacterium Lactobacillus reuteri on white spot lesion development in orthodontic patients and their data suggest that daily intake of probiotic lozenges containing L. reuteri had no effect on the incidence of WSLs developed during treatment with fixed orthodontic appliances.

Matheus M. Pithon, Mariana J. DOS Santos, et al (2015) **[57]** concluded that the varnish with CPP–ACP was capable of reducing tooth enamel demineralization in patients who use orthodontic appliances and proved that varnish containing CPP–ACP was shown to be more effective than the conventional varnish used to prevent white spot lesions around orthodontic brackets.

D. G. A. Nelson, w. L. Jongebloed, and j. Arends et at (1984) [(5)] assessed the surface layer morphologies of enamel treated either with an acidic silane fluoride lacquer, a neutral sodium fluoride varnish, or an acidulated phosphate fluoride (APF) gel were investigated using scanning electron microscopy (SEM). The particle size and agglomeration characteristics of each surface layer were assessed. The etching patterns of each topical fluoride agent were also determined and concluded that the effectiveness of a topical fluoride agent may be related to the changes it produces in surface enamel morphologies. The effectiveness of the APF gel seems to be related in part to the deep prism etch pits filled with extremely small (and reactive) CaF2 crystallites, which would not be as easily washed as CaF2 crystallites deposited on a smooth enamel surface. Other topical fluoride agents which produce an even etch (no pores) may not be expected to be as effective in reducing caries incidence.

E. K. Basdra et al (1996) [(9)] examined two fluoride-releasing orthodontic agents with respect to fluoride release, enamel demineralization inhibition, as well as alterations observed on the enamel surface after their use. Scanning electron microscopy of the enamel surface revealed particle depositions of micro globular form, after the examined adhesives were used. These particles most likely represent deposition of calcium fluoride, a salt with clearly cariostatic properties. The results of this in vitro study show that certain fluoride-releasing orthodontic bonding systems may provide an additional degree of safety against caries susceptibility in patients with fixed appliances for a limited period.

A. Corry, D. T. Millett, et al (2003) [27] compared the cariostatic potential of a resin modified glass ionomer cement (Fuji Ortho LC) to that of a resin control (Transbond) for bracket bonding and to compare the effect of extrinsic fluoride application on the cariostatic potential of each material and found that the creation of white spot inhibition could best be achieved by the use of a resin-modified glass ionomer cement, supplemented with fluoride exposure. The least protection was afforded by the composite control. The resin-modified glass ionomer cement alone and the composite with added fluoride demonstrated equivalent protection

Morten Fjeld, Bjørn Øgaard et al (2006) [63] investigated the effects of conventional etching with a 35% phosphoric etching gel and priming/bonding with Transbond XT primer/adhesive (3M Unitek, Monrovia, Calif), conditioning with 10% polyacrylic acid and bonding with a resin-modified glass ionomer cement (Fuji ORTHO LC, GC Corporation, Tokyo, Japan) or using a self-etching bonding system (Transbond Plus) and bonding with Transbond XT adhesive on the surface morphology of the enamel using a conventional scanning electron microscope. They found that Phosphoric acid etching produced a rough, etched surface with the typical honeycomb pattern. Bonding systems with self-etching primers or conditioners with polyacrylic acid might offer potential benefits compared with conventional acid etching and priming because of fewer irreversible changes to the enamel surface.

A Cochrane review on orthodontic adhesives by **Mandall Na, Miller R DT Marcusson A et al (2002)** **[53]** recommends the use of composite resin adhesives over GIC adhesives. Fluoride releasing composite resin has been developed, but releases less fluoride than RMGIC and GIC adhesives. Their survival time, however, is similar to conventional composites

Giulio Alessandri Bonetti, a Matteo Zanarini, et al (2011) **[40]** compared the modes of failure of uncoated and adhesive pre coated metal brackets by using the adhesive remnant index, and to assess the quality of the enamel surface after clean up by using the enamel damage index. Scanning electron microscope images of all labial enamel surfaces were taken at T0, T1, and T2, (before bonding -T0, after bracket removal-T1, and after clean up- T2) and these were evaluated according to the adhesive remnant index and the enamel damage index. They concluded that the differences between the enamel surfaces before bonding and after debonding were statistically significant, indicating that the debonding method tested in this study did not restore the enamel surface to its original condition, even though there was no clinically relevant damage to the enamel.

O.E. Eissaa, E.M. El-Shourbagy, S.A. Ghobashy et al (2013) **[66]** evaluated the in vivo effects of the fluoride releasing Transbond_ Plus Color Change Adhesive in reducing enamel demineralization around orthodontic brackets and compare it with non-fluoride releasing Transbond_ XT using SEM and EDX. They concluded that SEM observation and EDX examination showed that the fluoride releasing adhesive (Transbond_Plus Color Change Adhesive) resulted in a reduction of enamel demineralization around the bracket, when compared with conventional adhesive (Transbond_ XT).

Pritam Mohanty, Sridevi Padmanabhan (2014) (75), et al in their study, which aimed to evaluate the effects of Novamin® on enamel demineralization around the orthodontic brackets with EDX analysis concluded that EDX element analysis was found to be an efficient method to quantify the changes in mineral content of a sample during in vitro caries studies. It gives a validation of the effectiveness of in remineralization in the presence of mineral loss and justifies its use as an efficient method of preventing white spot lesions during orthodontic treatment.

Artun et al (2014) (7) studied the positive replicas of enamel at the time of debonding using scanning electron microscopy (SEM). The appearance of the lesion had changed from chalky white at the time of debonding to a more of diffuse opacity , particularly in the peripheral parts . Light microscopic studies indicated that enamel surfaces remineralize relatively rapidly after exposure to saliva

Monika Machoy, Julia Seeliger, et al (2016) (62) in their study aimed to examine the enamel surface after the completion of orthodontic treatment for the presence of elements recognized as toxic to the human body using scanning electron microscope (SEM) and EDS (Energy-Dispersive X-ray Spectroscopy) was used to identify the elements. X-ray microanalyzer (EDS) enabled analyzing the chemical composition of the test sample in the selected micro area of its surface—in this case within the crown and the composite material. The SEM-EDS analysis showed also the presence of silicone as a residue on the enamel after the orthodontic treatment. As silicone carcinogenicity end enzymes-inductive effects in mammals are brightly discussed and lately investigated it is very important to extend the researches in the dental industry. The silicones are regularly used in dentistry, especially in Endodontics as sealants, in prosthetics and orthodontics as impression materials, and in craniofacial surgery as internal implants. If the long-term studies give proof of silicone toxicity in human organisms, it will be essential to change the whole methodology of treatment in many fields of medicine. Metal ions, including aluminum, are present on the enamel surface after the completion of orthodontic treatment. The presence of aluminum was detected after cleaning the enamel using a polisher with aluminum oxides. Other methods for cleaning the enamel after orthodontic treatment should be assessed for the presence of residues of aluminum and other heavy metals. The problem of exposure of patients to adverse effects of heavy metals during and after orthodontic treatment should be explored and thoroughly investigated because of their potentially harmful effects on human health.

Eyup Burak Kucuk, Siddik Malkoc et al (2016) **[33]** did micro CT evaluation of WSL remineralization in various procedures and concluded that GC Tooth Mousse and Clinpro 5000 were more effective in remineralization of white spot lesions than sodium fluoride solution and artificial saliva. They can be preferred for clinical use. Microcomputed tomography is a novel and effective method that shows promise in accurately evaluating white spot lesions and remineralization.

Tahreh Baherimoghadam, Sahar Akbarian et al (2016) **[88]** stated that there is a significant increase in the number of enamel crack with the use of adhesion promoters but conservative debonding methods for decreasing enamel damages would be necessary.

Materials and Methods

MATERIALS AND METHODS

Patients who reported to the Department of orthodontics and dentofacial orthopedics were screened for the study. Patients in the age group of 13 to 25 years were included in the study as their oral hygiene is relatively poor. Five patients recruited for the study. This research was approved by the research and the ethical committee.

The criteria for inclusion were;

1. Subjects who required all first premolar extractions for orthodontic treatment,
2. All first premolars fully erupted with an intact buccal surface, and
3. No clinical evidence of any demineralized lesions or fluorosis.

The study and controls sides were randomly allotted on either side of both arches. Therefore, each patient served as his/her own control. Hence, the benefit of randomization was achieved through the split-mouth design. This distribution allowed the same environment for all teeth. The study was carried out for 60 days i.e. T1 in mandible and 90 days i.e. T2 in maxilla.

ARMEMENTARIUM:

1. Etchant
2. Bonding agent
3. Composite
4. Brackets
5. Arch wires

6. Clinpro XT
7. Light curing unit
8. Debonding pliers
9. Spatula
10. Modules

All patients received treatment with the light curable fluoride varnish (3M - Clinpro XT) after conventional bonding procedure on the experimental side.

PROCEDURE FOR EXPERIMENTAL SIDE:

After cleaning the teeth with non-fluoridated pumice paste, the enamel was etched for 30 seconds with 37% orthophosphoric acid and then rinsed for 10 seconds. Each tooth was dried with a stream of air until a chalky white appearance was observed. Transbond XT primer (3MUnitek) was applied to the etched surfaces in a thin, uniform coat. Then, Transbond XT light-cured adhesive paste (3M Unitek) was applied to the bracket base and placed against the enamel surface. A bracket placement pliers were used to hold and keep the bracket in position on the center of the enamel surface and excess adhesive around the bracket base was removed and light cured for 20 seconds with 3M Elipor light cure unit. Clinpro XT is available as a 2-paste system. Appropriate amount was taken and mixed into a thin layer and applied on the buccal surfaces of the 1^{st} premolar surrounding the bracket on the experimental side and then light cured for 20 seconds as per the manufacturer's instructions. All patients were instructed not to take food or water for 1 hour and not to brush their teeth for up to 6 hours. Routine oral hygiene instructions were given to each patient to maintain satisfactory oral hygiene, and non-fluoridated toothpaste (Himalaya active fresh gel) was advised during the study period.

PROCEDURE FOR THE CONTROL SIDE:

The premolars in the control group was etched for 30 seconds with 37% orthophosphoric acid and then rinsed for 10 seconds. Each tooth was dried with a stream of air until a chalky white appearance was observed. Transbond XT primer (3MUnitek) was applied to the etched surfaces in a thin, uniform coat. Then, Transbond XT light-cured adhesive paste (3M Unitek) was applied on the bracket base and light cured Excess adhesive around the bracket base was removed, and then the premolars (controls) were light-cured for a total of 20 seconds as described above.

Standard pre-adjusted edgewise stainless steel brackets (0.022" slot AO brackets with Roth prescription) were bonded on the labial aspect in both experimental and control sides. 0.014 NiTi wires were placed immediately after curing.

At the end of each time period T1=60 days and T2 = 90 days, brackets were debonded. A careful de-bonding procedure was done to ensure that there were no enamel micro fractures.

The teeth were extracted carefully by using a gauze to cover the crown surface of the tooth and the forceps were placed sub gingival to avoid any damage that may cause to the tooth during extraction. Extraction was carried out by a single operator. At T1 i.e. 60 days' time period mandibular 1st premolars were extracted and at T2, i.e. 90 days' time period 1st premolars of maxillary arch were extracted.

These specimens were then stored in a deionized distilled water (MILLI -Q water)

SEM sample preparation. –

SEM specimens were attached to aluminium stubs with a fast-curing epoxy resin. The samples were then sputtered with a 10 - 12-nm-thick layer of gold in a Poloron -Range sputter apparatus. The specimens were then examined with a JEOL JSM-35C scanning electron microscope operated at 20kV. The tooth was mounted such that the buccal surface was normal to the incident electron beam and photomicrographs were obtained.

EDAX sample preparation. –

After the SEM analysis, the samples were then shifted to EADX detector (Oxford instruments X-MAS LN2; 139EV) Free, Peltier Cooled 139Ev). The buccal surface of the teeth were sputtered with gold material as tooth is a on conductive agent .The buccal surface of the teeth was examined carefully at the gingival (Location I) and the occlusal (Location II) portion of the orthodontic bracket area to obtain the representative microphotographs and elemental analysis.

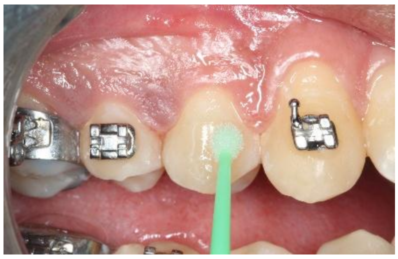
STATISTICAL ANALYSIS

Data entry and analysis were performed using the SPSS statistical package for social service Version 20 (IBM, IL USA). Descriptive statistical analysis of all the elements and for each of the predictor values were done. The predictor values were the number of days, study samples / control samples. The outcome variables were the measures of elements like Oxygen, Sodium Aluminum, Phosphorus, Chlorine, Calcium, Fluoride, Magnesium, Silicon, and Iron expressed as weight percentage as well as the Calcium - Phosphorus ratio. One way analysis of variance (ANOVA) was used to differentiate between the mean and standard deviation between the groups and within the groups. The statistical significance level was established at P value ≤ 0.05.

Figures

Before bonding

Etching of the enamel

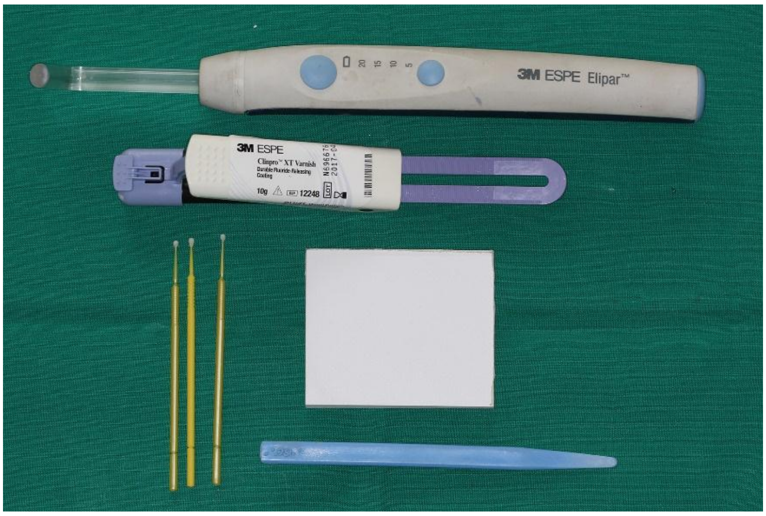


Primer application

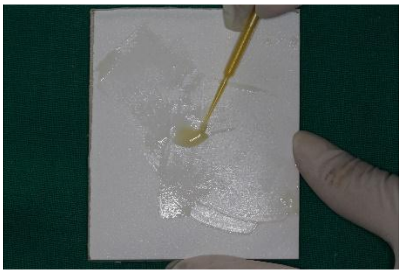
Bracket positioning and removal of the excess

Light curing

Post -bonding



Armamentarium for varnish aplication

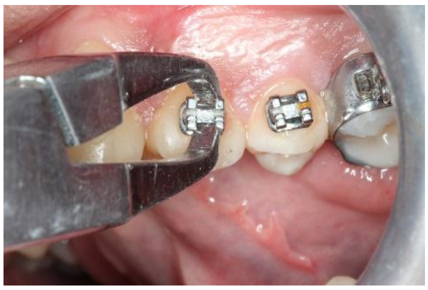


Mixing of the varnish

Application of the varnish
around the bracket

Light curing the varnish

Debonding the bracket at 60 days



Debonding the bracket at 90 days

Post debonding

Extraction

Figure 1. Procedure of fluoride varnish application followed by extraction

Results

RESULTS

SCANNING ELECTRON MICROSCOPY ANALYSIS:

Scanning electron microscopy examination of the enamel surface of the study group revealed almost normal topographic features of enamel. In some cases, there were globules of calcium fluoride like material irregularly distributed near the bracket area over the enamel surface. This globular calcification was arranged around enamel rod peripheries where as some rods were occluded as shown in fig 2 and fig 4 at 60 and 90 days representing the study samples.

Morphological changes suggesting enamel remineralization were observed near the composite remnants. In some specimens, globules of calcium fluoride like material were detected, resulting in areas of enamel remineralization with occlusion of enamel rods and repair as shown in fig 2 and fig 4.

SEM observation of the enamel surface in the control group revealed roughened enamel surface with multiple areas of enamel erosion Various patterns of enamel decalcification were observed in the form of open focal holes and demineralization of enamel rod core were noted. Highly roughened enamel surface and erosion of enamel rod cores were noted as shown fig 3 and fig 5 at 60 days and 90 days representing the control sample.

ELECTRON DISPENSIVE X-RAY ANALYSIS:

Descriptive statistics for the 10 elements and calcium to phosphorus ratio (Ca-P ratio) in location I (gingival margin to the gingival border of the bracket) for 60 days for both study and control samples has been given in Table I and Graph I with mean and standard deviation.

An increase in weight percentage of elements phosphorus, calcium, fluoride and the Ca-P ratio and decrease in weight percentage of elements oxygen, aluminum and silicon were noted in the study group when compared to that of control group at 60 days in location I.

Descriptive statistics for the 10 elements and calcium to phosphorus ratio (Ca-P ratio) in location I (gingival margin to the gingival border of the bracket) for 90 days for both study and control has been given in Table II and Graph II with mean and standard deviation.

An increase in weight percentage of elements sodium, aluminum fluoride, magnesium, silicon and iron were noted and a decrease in weight percentage of elements phosphorus, calcium and Ca-P ratio were noted in the study sample when compared to that of control sample at 90 days in location I.

Descriptive statistics for the 10 elements and calcium to phosphorus ratio (Ca-P ratio) in location II (at incisal part of the bracket to the occlusal margin) for 60 days for both study and control has been given in Table III and Graph III with mean and standard deviation

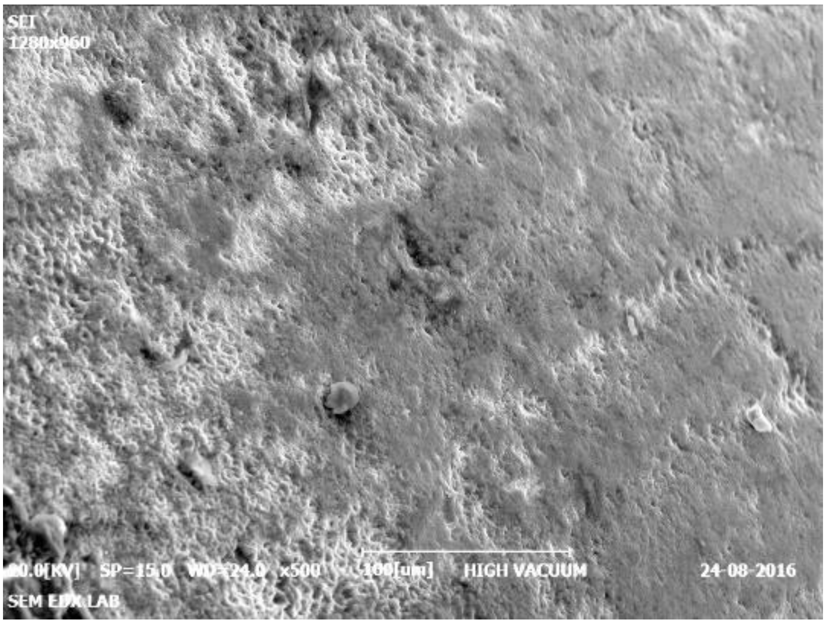
An increase in weight percentage of elements oxygen, sodium, aluminum, fluoride and silicon and decrease in weight percentage of phosphorus, calcium and Ca-P ratio were noted in the study samples when compared to that of control samples at 60 days in location II.

Descriptive statistics for the 10 elements and calcium to phosphorus ratio (Ca-P ratio) in location II (at incisal part of the bracket to the occlusal margin) for 90 days for both study and control has been given in Table IV and Graph IV with mean and standard deviation.

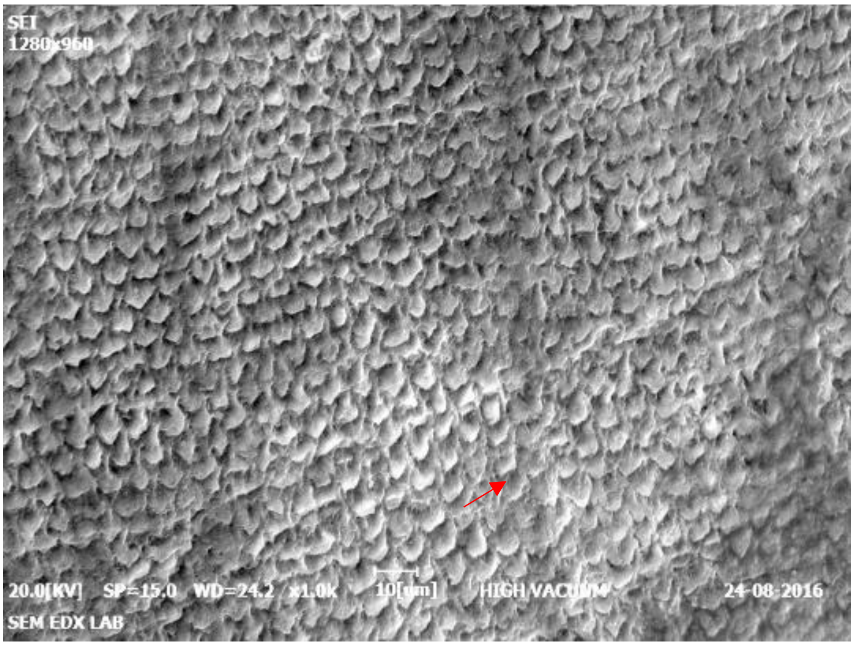
An increase in weight percentage of elements oxygen, aluminum, fluoride and silicon and decrease in weight percentage of phosphorus, calcium and Ca-P ratio were noted in the study samples when compared to that of control samples 90 days in location II.

The comparison of various elements at 60 days and 90 days for within and between the groups were performed using one way analysis of variance (ANOVA) and is given in Table V and Graph V, Graph and VI. Each element is represented in weight percentage in both the groups. The results show that there is a significance difference in sodium, aluminum, phosphorus, calcium, fluoride, magnesium and iron. Whereas oxygen, chlorine silicon and Calcium -Phosphorus ratio is statistically insignificant.

A statistically significant increase in the weight percentage of sodium, aluminum, fluoride, magnesium and iron were noted in the study sample when compared to that of the control sample. The difference in weight percentage of elements oxygen, chlorine, silicon and Calcium -Phosphorus ratio was statistically insignificant.

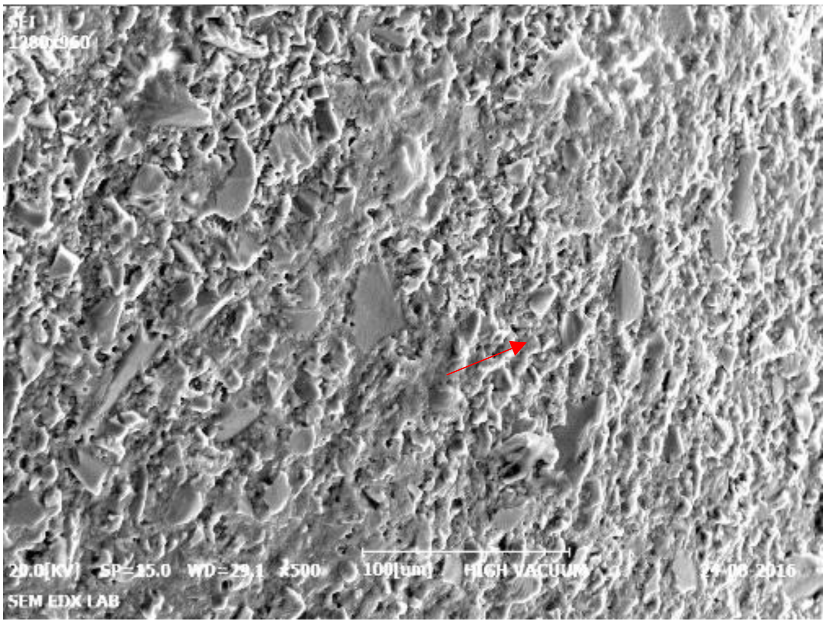


Relatively smooth enamel surface suggesting of enamel remineralization (arrows).(X500)

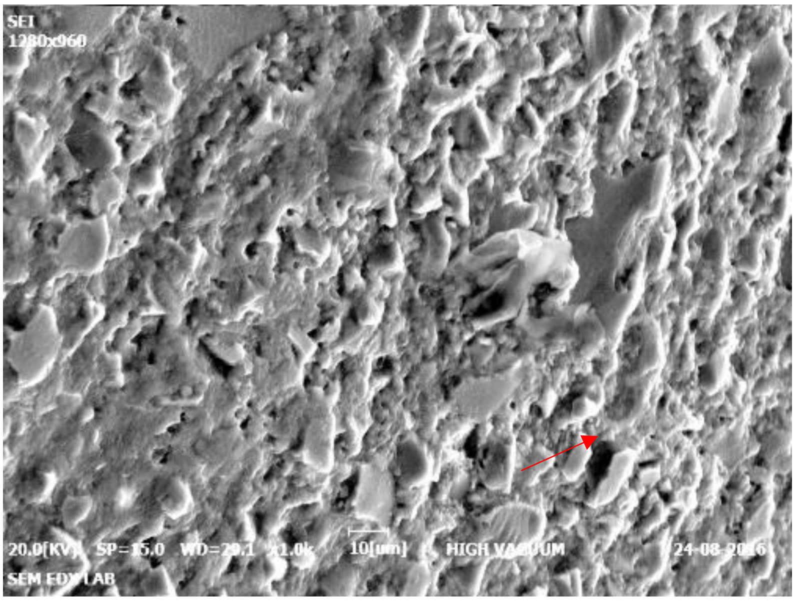


Occluded enamel rods and calcium fluoride like globules are noted. (arrows).
(X 1.0k)

Figure 2: SEM images at 60 days in study group

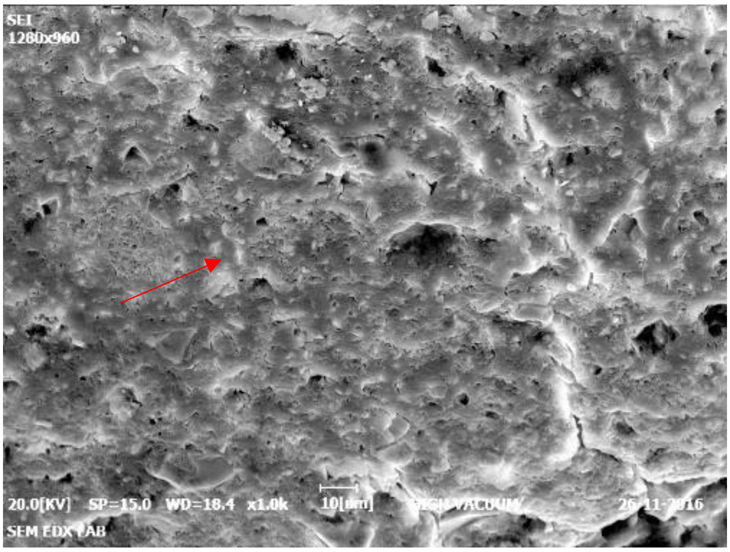


Multiple areas of enamel erosion represented by arrow
(X500)

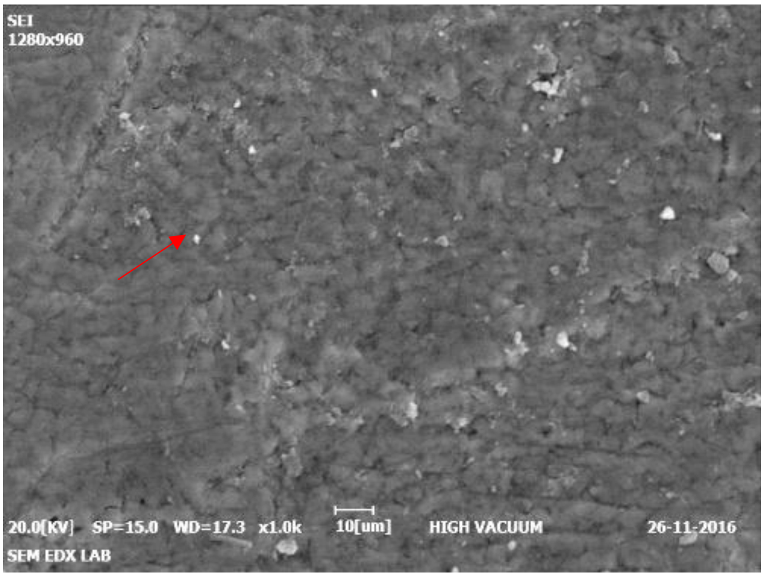


Multiple areas of enamel erosion represented by arrow
(X1.0k)

Figure 3 : SEM images at 60 days in control group

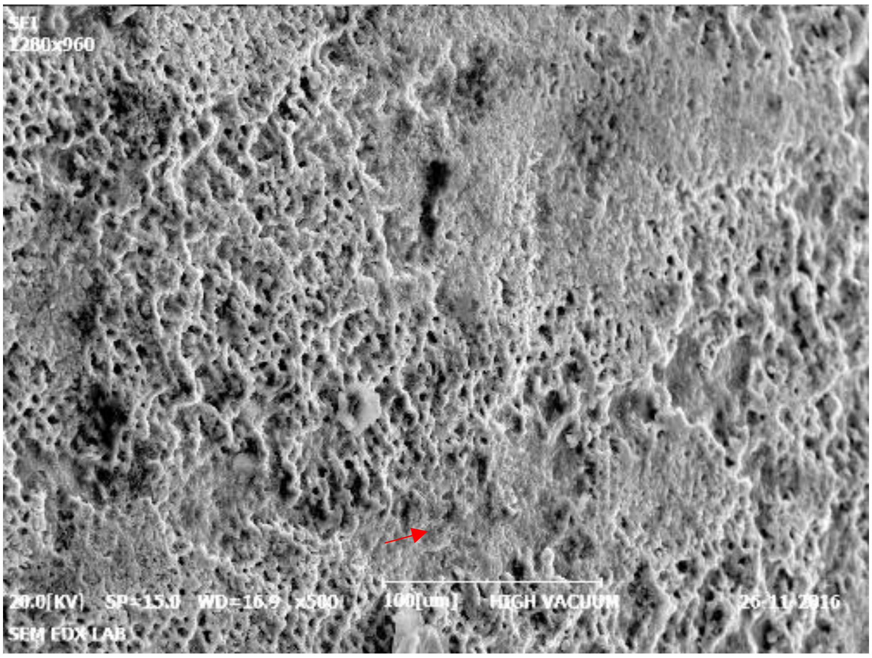


Morphological changes suggestive of enamel remineralization is represented by arrows
(X 500)

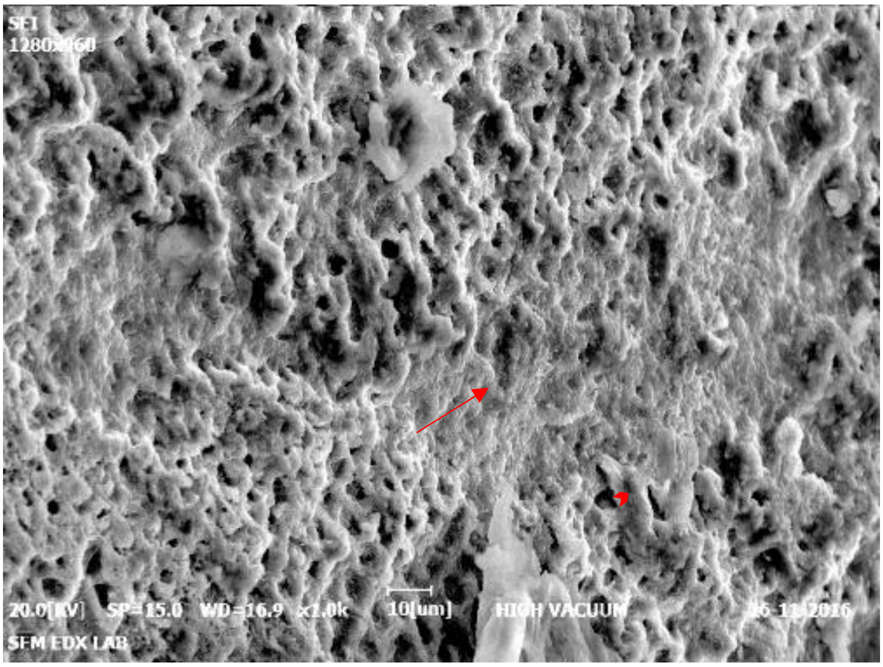


Relatively smooth surface topographic features of enamel with an appearance suggestive of enamel remineralization(X1.0K)

Figure 4: SEM images at 90 days in study group

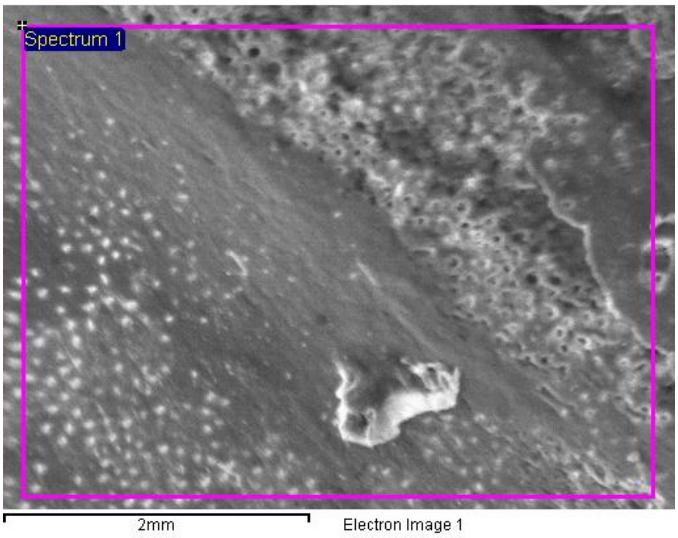


Multiple areas of enamel erosion represented by arrow (x500)



Multiple areas of enamel erosion suggestive of enamel demineralization (X1.0K)

Figure 5: SEM images at 90 days in control group



Spectrum processing :

No peaks omitted

Processing option: All elements analyzed (Normalized)

Number of iterations = 5

Standard :

O SiO2 1-Jun-1999 12:00 AM

P GaP 1-Jun-1999 12:00 AM

Ca Wollastonite 1-Jun-1999 12:00 AM

Element	Weight%	Atomic%
O K	65.29	80.95
P K	12.86	8.23
Ca K	21.86	10.82
Totals	100.00	

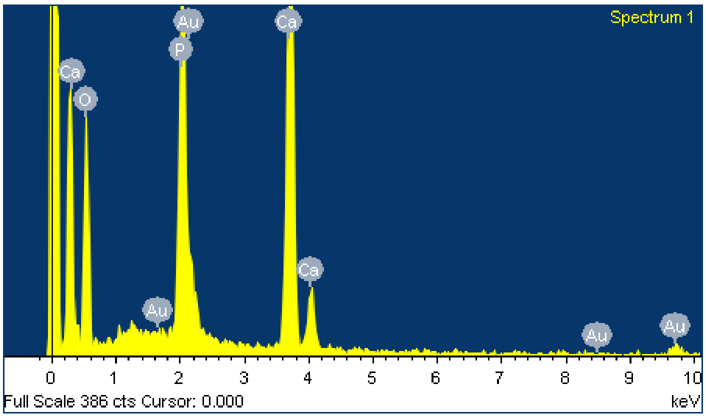


Figure 6 : EDAX result for the control group

Spectrum processing :

No peaks omitted

Processing option: All elements analyzed (Normalized)

Number of iterations = 5

Standard :

O SiO2 1-Jun-1999 12:00 AM

F MgF2 1-Jun-1999 12:00 AM

Na Albite 1-Jun-1999 12:00 AM

Mg MgO 1-Jun-1999 12:00 AM

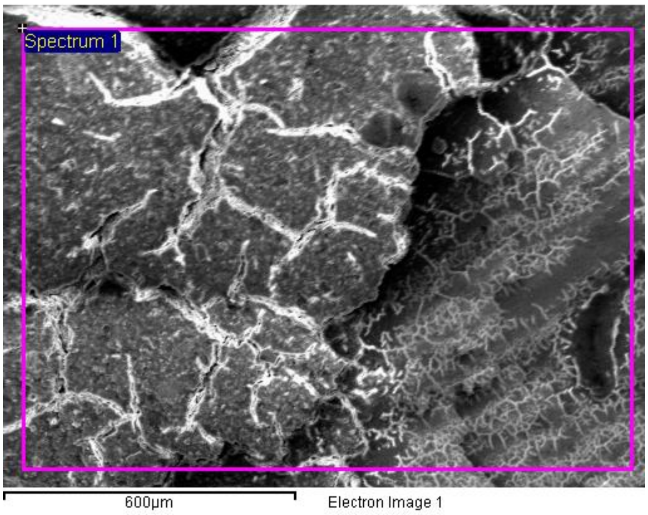
Al Al2O3 1-Jun-1999 12:00 AM

Si SiO2 1-Jun-1999 12:00 AM

P GaP 1-Jun-1999 12:00 AM

Cl KCl 1-Jun-1999 12:00 AM

Ca Wollastonite 1-Jun-1999 12:00 AM



Element	Weight%	Atomic%
O K	55.35	70.03
F K	5.86	6.25
Na K	1.16	1.02
Mg K	0.67	0.55
Al K	4.49	3.36
Si K	4.12	2.97
P K	9.84	6.43
Cl K	0.51	0.29
Ca K	18.00	9.09
Totals	100.00	

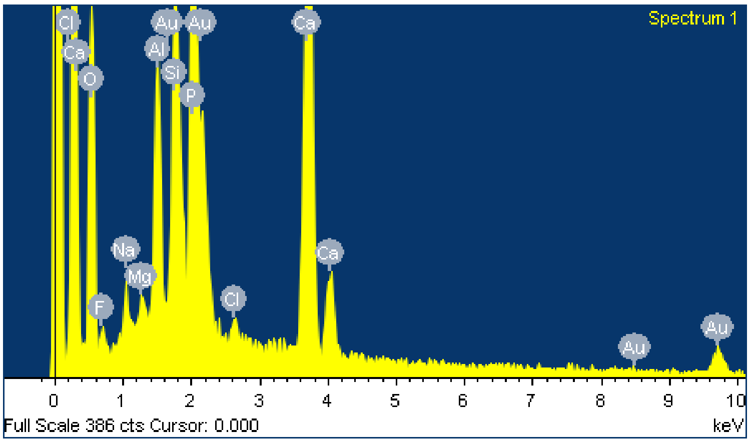


Figure 7 : EDAX result for the study group

TABLE I : Descriptive statistics for elements at location I (gingival margin to the gingival border of the bracket) at 60days

	STUDY	CONTROL
	MEAN ±SD	MEAN±SD
Oxygen	51.612±4.871	54.172±4.827
Sodium	0.832±0.687	0.954±0.365
Aluminium	1.372±1.422	2.468±5.413
Phosphorus	11.394±6.742	8.688±8.073
Chlorine	1.922±3.123	0.56±0.705
Calcium	25.852±7.793	21.272±11.732
Fluoride	2.87±2.411	1.134±1.642
Magnesium	0.00	0.17±0.244
Silicon	4.276±6.501	10.49±7.4
Iron	0.00	0.00
CaP-ratio	158.196±88.959	114.437±105.83

TABLE II: Descriptive statistics for elements at Location I (gingival margin to the gingival border of the bracket) at 90days

	STUDY	CONTROL
	MEAN±SD	MEAN±SD
Oxygen	54.408 ± 4.795	55.642 ± 10.473
Sodium	1.478 ± 0.995	0.69 ± 1.125
Aluminium	4.496 ± 4.109	0
Phosphorus	5.708 ± 4.027	14.528 ± 2.674
Chlorine	0.966 ± 0.572	1.044 ± 0.902
Calcium	13.942 ± 7.861	27.764 ± 8.078
Fluoride	6.66 ± 4.954	0.00
Magnesium	0.476 ± 0.437	0.00
Silicon	11.658 ± 7.071	2.322 ± 3.908
Iron	0.1 ± 0.223	0.00
CaP-ratio	162.25 ± 98.775	188.453 ± 21.659

TABLE III: Descriptive statistics for elements at location II(incisal part of the bracket to the occlusal margin) at 60days

	STUDY	CONTROL
	MEAN±SD	MEAN±SD
Oxygen	51.752±10.981	42.762±11.847
Sodium	1.886±1.023	0.454±0.416
Aluminium	5.32±5.651	0.022±0.049
Phosphorus	9.628±6.012	17.414±2.96
Chlorine	0.666±0.62	0.386±0.363
Calcium	17.988±13.767	36.05±11.219
Fluoride	5.716±4.663	0.434±0.617
Magnesium	0.182±0.353	0.04±0.058
Silicon	6.61±6.932	2.436±4.728
Iron	0.1±0.223	0.00
CaP-ratio	181.55±42.891	203.198±29.273

TABLE IV: Descriptive statistics for elements at location II (incisal part of the bracket to the occlusal margin)at 90 days

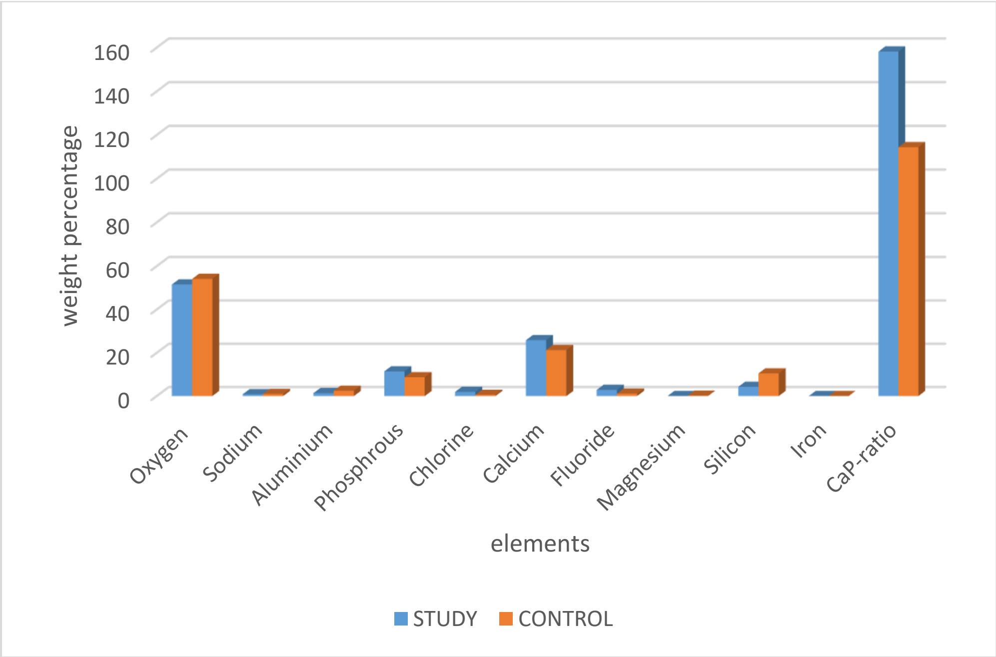
	STUDY	CONTROL
	MEAN±SD	MEAN±SD
Oxygen	57.074±5.148	57.91±5.94
Sodium	1.16±1.166	0.44±0.71
Aluminium	3.916±2.893	0.00
Phosphorus	6.5±4.697	13.18±2.54
Chlorine	0.854±0.315	0.61±0.47
Calcium	12.16±6.715	23.19±3.88
Fluoride	5.728±3.381	0.00
Magnesium	0.264±0.361	0.00
Silicon	12.02±13.968	1.86±2.73
Iron	0.324±0.472	0.00
CaP-ratio	151.601±88.683	176.82±10.07

Table V : Comparison of various elements between and within the groups

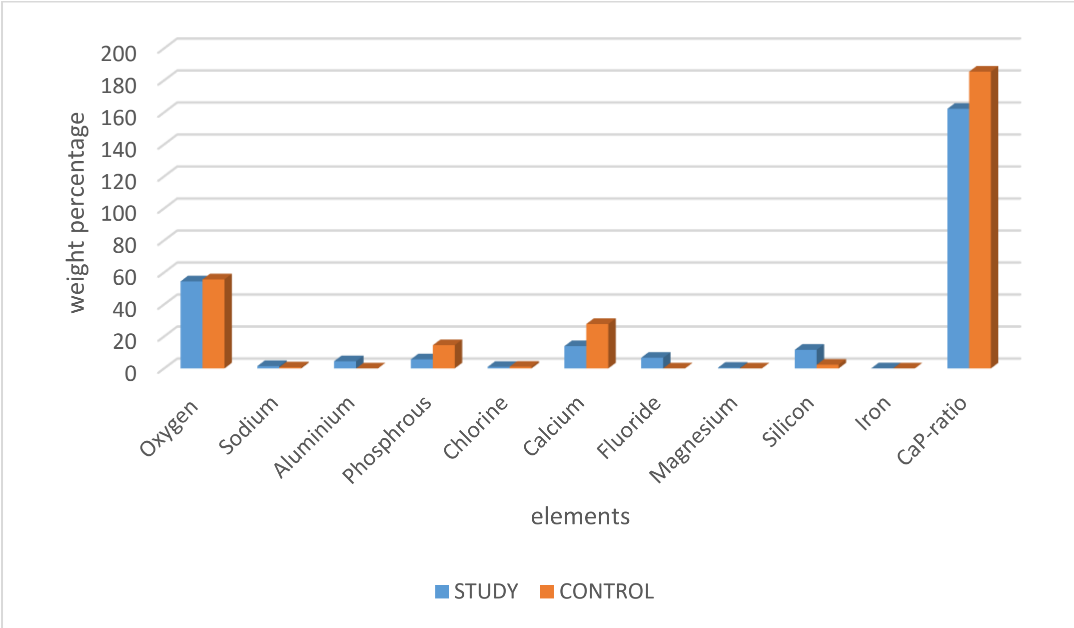
	STUDY		CONTROL		p value
	60days	90days	60days	90days	
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	
OXYGEN	51.68 ± 8.00	55.74 ± 4.89	48.46 ± 10.43	56.77 ± 8.11	0.69
SODIUM	1.35 ± 0.99	1.31± 1.03	0.70 ± 0.45	0.56 ± 0.89	0.013
ALUMINIUM	3.34 ± 4.40	4.20 ± 3.36	1.24 ± 3.83	0.00	0.005
PHOSPHROUS	10.51 ± 6.09	6.10 ± 4.14	13.05 ± 7.34	13.85 ± 2.56	0.005
CHLORINE	1.29 ± 2.22	0.91± 0.43	0.47 ± 0.53	0.83 ± 0.71	0.243
CALCIUM	21.9 ± 11.33	13.05 ± 6.95	28.66 ± 13.33	25.48 ± 6.44	0.005
FLUORIDE	4.29 ± 3.80	6.19 ± 4.02	0.78 ± 1.22	0.00	0.00
MAGNESIUM	0.09 ± 0.25	0.37 ± 0.39	0.10 ± 0.18	0.00	0.042
SILICON	5.44 ± 6.45	11.83 ±10.43	6.46 ± 11.72	2.09 ± 3.19	0.128
IRON	0.05 ± 0.15	0.21 ± 0.36	0.00	0.00	0.049
CA-P RATIO	169.87 ± 66.98	156.92 ± 88.67	158.81 ± 86.87	182.63 ± 17.06	0.742

P value ≤ 0.05 considered statistically significant

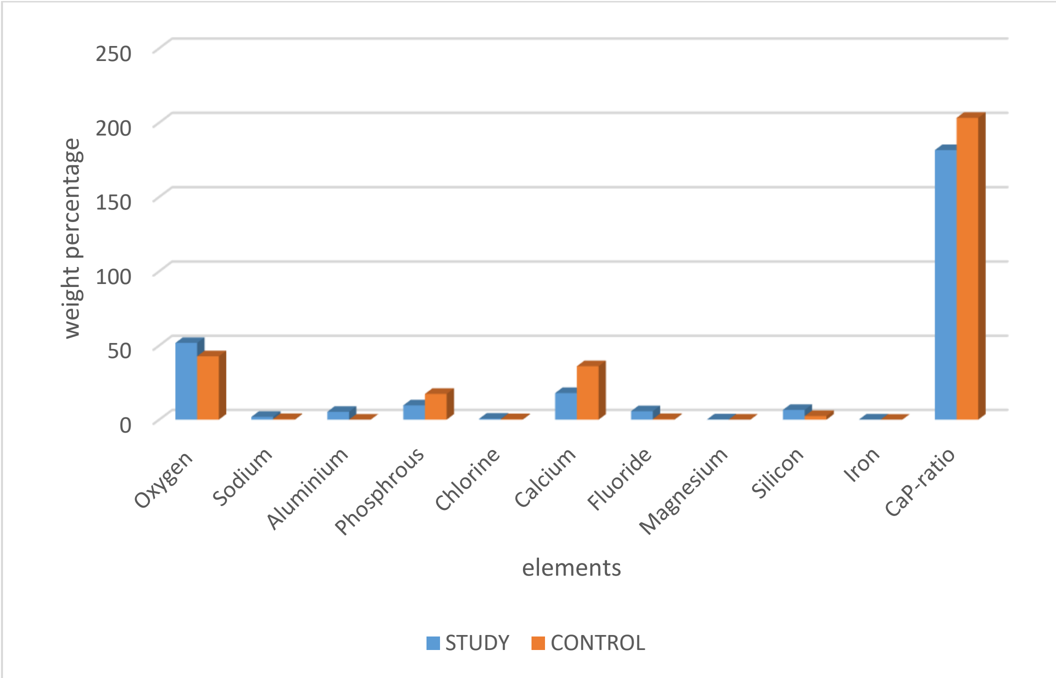
GRAPH – I: Descriptive statistics for elements at location I (gingival margin to the gingival border of the bracket) at 60days



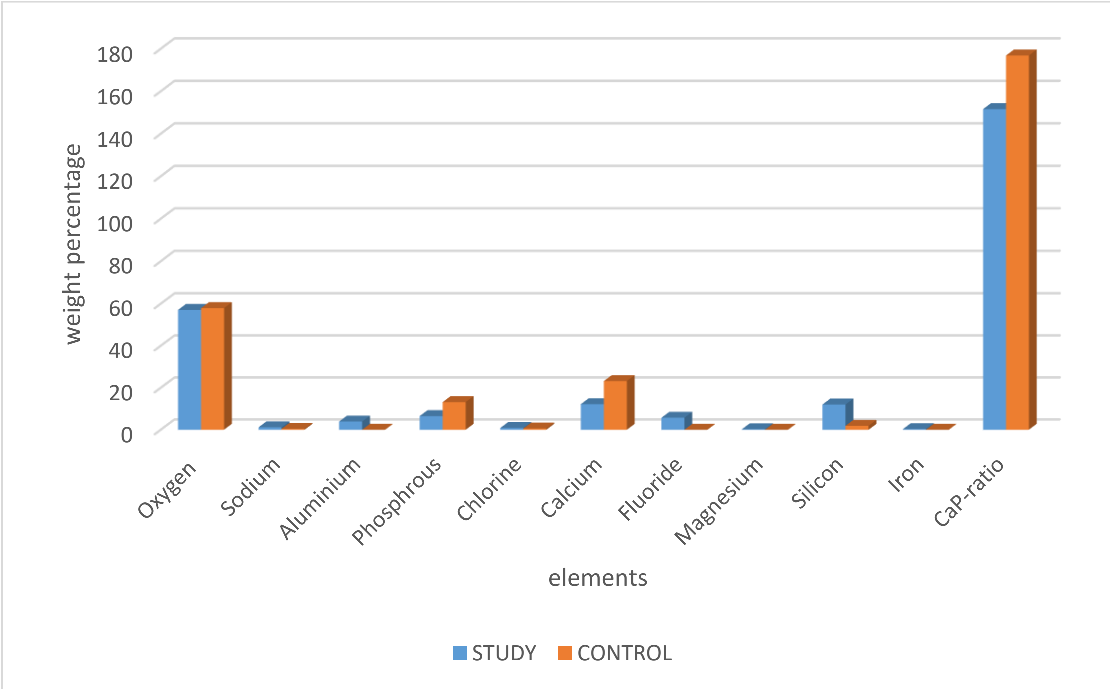
GRAPH – II : Descriptive statistics for elements at Location I (gingival margin to the gingival border of the bracket) at 90days



GRAPH III : Descriptive statistics for elements at location II(incisal part of the bracket to the occlusal margin) at 60days

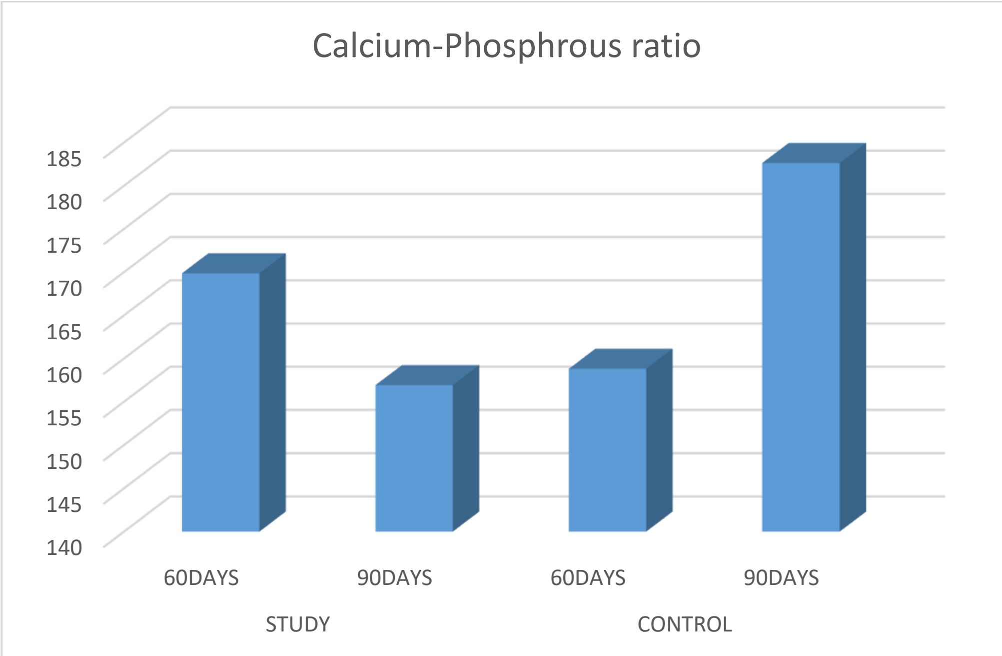


GRAPH IV : Descriptive statistics for elements at location II (incisal part of the bracket to the occlusal margin)at 90 days



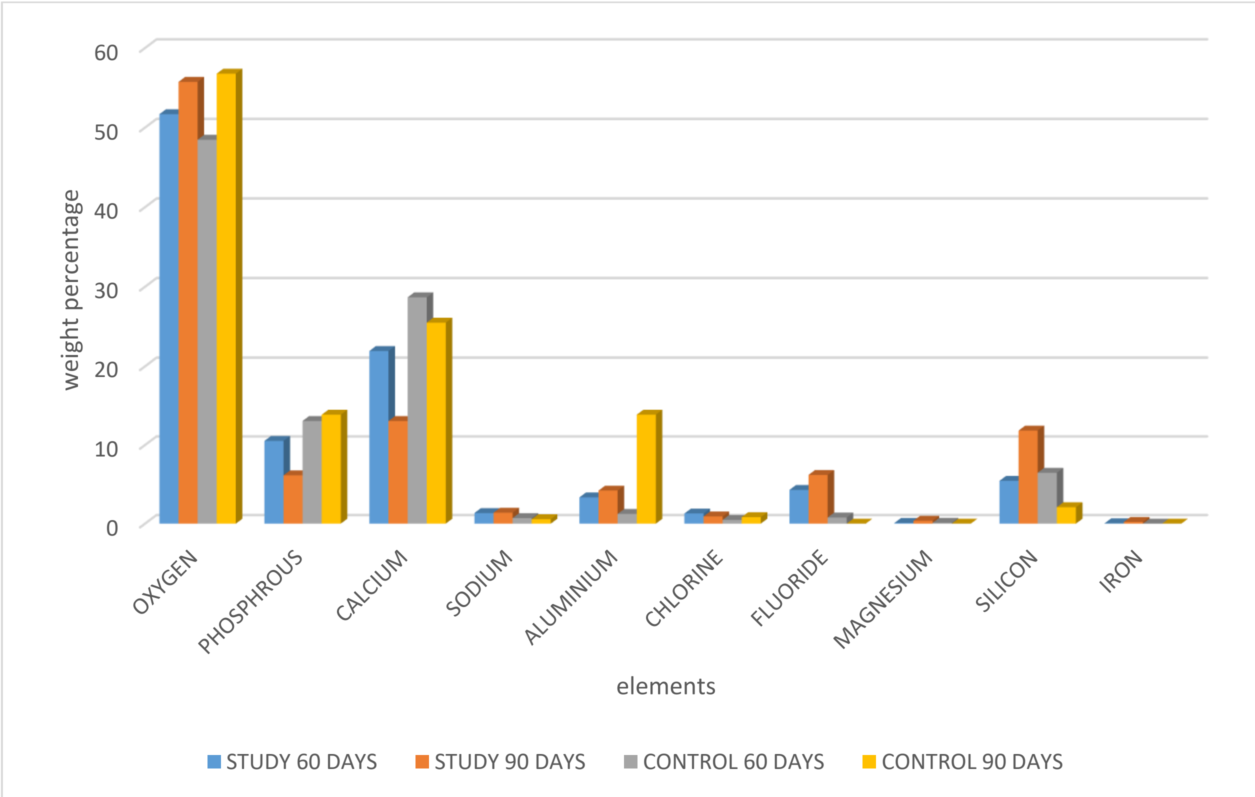
Comparison of Calcium- Phosphorous ratio between and within the groups

GRAPH V



Comparison of various elements between and within the groups

GRAPH VI



Discussion

DISCUSSION

The presence of orthodontic attachments alters the oral environment enhancing plaque accumulation around the brackets resulting in enamel demineralization. Prevention of enamel demineralization is one of the major concern of the orthodontist in addition to delivering high quality treatment. Various methods have been implemented to prevent and control the demineralization of the enamel during orthodontic treatment which causes unaesthetic appearance of the dentition by forming white spots. It’s a general consensus that patients and parents expectations at the end of orthodontic treatment is not only to improve oral health but also to enhance their self-esteem and social life. But this can be threatened by the unsightly appearance of the sub surface lesion at the time of debond.

White Spot Lesions are frequently encountered in orthodontic patients with poor oral hygiene. This damaged enamel shows up most frequently as a white, opaque area outlining the site of bracket bonding. [(81)] Considerable research has been devoted to fluoride delivery methods that reduce or minimize enamel demineralization and favors remineralization in orthodontic patients. The prevention of white spot lesions and enamel demineralization is still one of the major concerns for the clinicians. Up to 97% of patients undergoing orthodontic treatment are affected by white spot lesions, given that studies show that restoration of these lesions were very difficult. As patient compliance varies it is imperative to take all preventative measures.

One of the most successful ways of preventing and controlling WSL's during orthodontic treatment is through fluoride varnish application. The use of fluoride varnish is continuously being investigated in search for an effective method to prevent white spot lesions, especially for ease of professional application in long-term clinical situations. It has been widely accepted that fluoride varnishes can inhibit enamel demineralization around orthodontic bracket area. The light curable fluoride varnish (LCFV) used in our study is useful in reducing dentine hypersensitivity but literature evidence is limited in relation to its role in preventing enamel demineralization.

In our study, the effect of LCFV (light cured fluoride varnish) was observed for a period of 3 months without interfering with the orthodontic treatment process for 60, and 90 days. The uniqueness of this in-vivo study is that it is designed so that the oral environment was simulated which is routinely experienced in clinical settings. Elastic modules used in this study were routinely used during treatment with fixed appliances. The modules and the elastic ties around orthodontic bracket make plaque removal difficult with regular brushing. Therefore, the need for effective means of fluoride delivery around orthodontic bracket to prevent enamel demineralization is mandatory. According to Seppa et al plaque did not prevent fluoride uptake from varnish and it is suggested to promote fluoride uptake by enamel. This was supported by Bruun and Stolize (1976), Charlton et al (1974), Turtola 1977 and Weatherell et al (1972) [70]. There is also evidence that effect of fluoride ions were pronounced in the presence of fermenting plaque.

Various split-mouth study designs have evaluated the cariostatic effect of fluoride releasing material and in our study the subjects were randomly divided into 2 groups, the study group and the control group. The premolars n the study side received the LCFV application. Prior clinical and radiographic examinations showed that the patients were equivalent regarding caries risk or activity.

Patients were advised not to have any form of external fluoride supplement and were asked to refrain from any fluoride rinses and any form of fluoride treatment for the entire duration of the study to reduce the bias.

In the present study, upper premolars were subjected to the maximum duration of the experiment. Ogaard et al. [(68)] suggested a relationship between resistance to white spot formation and the rate of salivary flow. Because of the influence of salivary glands and gravity the lower dentition will have a higher degree of salivary contact. For this reason, extraction of first premolars in lower jaw were done at 60 days and upper premolars were done at 90 days. This was also based upon the fact that maxilla is more prone to white spot lesion than mandible.

The LCFV used in this study is a RMGI (resin modified glass ionomer) is methacrylate modified polyalkenoic acid. The primary components of the liquid consist of polyalkenoic acid, HEMA (2-hydroxethylmethacrylate), water and initiators which included camphorquinone and calcium glycerophosphate. The paste is a combination of HEMA, BIS-GMA water, initiators and fluoroaluminosilicate glass (FAS glass).

In-vitro testing has shown that the RMGI chemistry in Clinpro™ XT Varnish provides a fluoride burst that is released during the first few days after application and long term sustained release of fluoride from the coating. The fluoride present in fluoroaluminosilicate glass particles (FAS glass) and reaction at the surface provides a spontaneous release, while the bulk of FAS glass particles act as a reservoir for sustained release of fluoride.

A 2 and 3-month experimental period was used in the present study and there was a statistical increase in weight percentage of fluoride in 90 days and 60 days in study group when compared to that of the control group. In the study group at 60 days, fluoride release was around 2.87 ± 2.41 by weight percentage in location I (gingival margin to the gingival border of the bracket) and 53.71 ± 0.46 by weight percentage in location II (incisal part of the bracket to the occlusal margin) and at 90 days the fluoride release was 6.66 ± 4.95 by weight percentage in location I and 5.17 ± 4.66 by weight percentage in location II indicating an increase in fluoride weight percentage Where as in the control group at 60 days the amount of fluoride was 1.13 ± 1.64 by weight percentage in location I and 0.434 ± 0.61by weight percentage in location II and at 90 days there was no fluoride present in both location I and location II indicating decrease in fluoride weight percentage. This showed fluoride release was continuous for 90 days' duration of the study. SEM examination reveals that in the control group enamel demineralization occurs within 60 days i.e. 8 weeks (fig3). This finding was in accordance with the study of Ogaard et al [(68)] and O. Reilly et al [(67)] who reported that white spot lesions can occur within one month after bonding.

The use of light-cured and chemically cured sealants has been reported to prevent enamel demineralization around orthodontic brackets. Chemically cured sealants do not effectively and smoothly seal to the enamel surface because of oxygen inhibition of polymerization. (96,22) In-vitro studies of light - cured sealants have demonstrated that the light-cured unfilled resin provide more protection than chemically cured sealants. (46). This means that retention of the varnish on the tooth surface is an important factor in selecting a fluoride delivering method. Hence, in this study, we used a light curable fluoride varnish.

Clinpro XT, because it is light cured, it is advantageous; and moreover, the wear resistance of the varnish is relatively high that it can resist up to 5000 strokes of brushing (according to the manufacturer's claim; 3M ESPE Internal Data, www.3MESPE.com.au) before mechanical deformation. The resistance of the light curable fluoride varnish to brushing strokes was not evaluated in our study.

The amount of fluoride, which is necessary for the prevention of WSL or enamel demineralization has always been a debate in orthodontics. Basdra et al (9) explained in his study on fluoride release initially causing a burst effect would be more effective in preventing enamel demineralization. Contradicting this Linton et al (52) documented that rather than a high dose of fluoride i.e. almost 225ppm a small dose of 50ppm is more effective and stated that high concentration of fluoride block the surface layer by preventing penetration of calcium ions to subsurface layer (71). It seems that high doses of fluoride are useful in inhibiting lesion formation, and low doses are effective in remineralization and controlling the progress of the lesions. (79)

In-vitro testing has shown that Clinpro XT is effective in short term and long term fluoride release i.e. 24 hours to over 6 months. However, in vivo studies may differ from in vitro experiments. In our study, there was an increase in the fluoride weight percentage in study group compared at 60 days and 90 days, which shows a long-lasting fluoride release up to 90 days.

The cariostatic effect of fluoride varnish enhances by repeated application of the varnish [(32)] and various clinical regimens have been suggested to reduce carious lesion depth. It may be an annual application, [(91)] and a semi-annual application, [(11)] or monthly applications of fluoride varnish have also been proposed for orthodontic patients with a high risk of developing white spot lesions, or those who already have them. [(74)]. The results of a recent systematic review by Benson et al reported with moderate evidence that application of fluoride releasing varnish every 6 weeks during treatment is very effective [(12)] i.e. if orthodontic treatment is for 2 years then 16 application is required to achieve almost complete protection. Our study offers some evidence that light curable fluoride varnish may provide protection for up to 3 months or even longer, and may reduce the frequency to 4 to 6 applications for 2-year treatment period. Light curable fluoride varnish can decrease the application by threefold which is significant. The results of our study showed that there was a sustained fluoride release in 90 days in the study group (Table II and Table IV) compared to the control group indicating that fluoride varnish lasts up to 90 days. One of the limitation of our study is that fluoride release was evaluated only for a period of 90 days and long term effect of light curable fluoride releasing varnish was not verified and further studies are required to confirm the short term and long term effect of fluoride release by Clinpro XT.

Scanning electron microscopy analysis done on the enamel surface of the fluoride-releasing material in the study group revealed almost normal topographic features of enamel. As observed in SEM occlusion of enamel rods and repair of fractured enamel surface, by deposition of calcium fluoride crystals were suggestive enamel remineralization. These globular calcifications were arranged all around inter-rod region surrounding the enamel rods and as well as along enamel rod peripheries were noted in the study group. No such globules were noted in the control group samples. The observations in our study were similar to those reported by Nelson et al., Ogaard, and Bykyilmaz et al [74,12,64] after topical fluoride applications. Because of their resemblance in appearance to calcium fluoride (spherical globules), one could speculate that these particle depositions most likely represent calcium fluoride, a salt which clearly have cariostatic properties [70].

During acid attacks the calcium fluoride that has formed on the surface enamel may act as a potential reservoir [20] for slow fluoride ion release, which aids in remineralization of demineralized enamel and therefore acting as a barrier during acid attacks [70]. A large and more acid resistant calcium fluoride crystals were formed either during the initial phase or later phase. During the slow but regular exposure, there is a dissociation of fluoride ion happening from the calcium fluoride crystals that is then diffused to the pores in the enamel which may have occurred by incorporation into the hydroxy apatite crystals as fluorapatite during demineralization and remineralization process. These crystals act as a barrier which prevent the natural remineralization process of deposition of calcium and phosphorous onto the enamel surface from saliva. This is evident in our study as the amount of Calcium, Phosphorous and the Calcium -phosphorous ratio (CA -P ratio) were reduced in study sides when compared to that of the control group (Table V and Graph V). It is also noted that there was a decrease in deposition of calcium and phosphorus in the study group when compared to that of the control group in 90 days (Table V and Graph V). This is in accordance with the study of Ogaard, (71) who stated that the high dose of fluoride physically blocks the surface layer of enamel from penetrating calcium ions to subsurface layers. This decrease in calcium -phosphorus ratio can also be due to greater dental plaque accumulation and the patient's difficulty in cleaning this area (73).

Previous studies have reported that acid etching prior to enamel bonding is a possible causative factor in the decalcification associated with orthodontic treatment (9). More importantly, the acid-etched surface allows the less mineralized underlying enamel to be exposed to a potentially acidic microenvironment (73). Therefore, etched enamel exposed to cariogenic solutions, has been shown to be more severely affected than the unetched enamel (67). Adhesive resin used to bond brackets to the enamel can also cause enamel decalcification and polymeric structure of resins hosts a variety of microorganisms. Increases in bacterial accumulation have been reported at orthodontic bracket sites (67). Bacteria, not only adhere to, but also consume and colonize within, the composite resin (29). An increase in bacterial accumulation following orthodontic appliance placement has been observed (83). Different etching patterns and the bacterial accumulation in the control as well as the study group have not been evaluated in our study.

In the present study two locations were selected for the EDAX analysis as Location I (gingival margin to the gingival border of the bracket) and at Location II (incisal part of the bracket to the occlusal margin). This was based on the study of Gorelick et al. (1982) (42) and Øgaard (1989) (71) who stated that the enamel demineralization around the brackets is commonly seen on the buccal surfaces of teeth, especially in the gingival region.

EDAX is defined as a quantitative, and qualitative method for identification of chemical elements in a wide variety of samples. The present study analyzed the composition of elements like oxygen, sodium, aluminum phosphorous chlorine, calcium and fluoride, magnesium, silicon, iron and Calcium – phosphorous ratio. The calcium, phosphorous and fluoride, which is considered as the caries preventive agent (56) are present in considerable amount in our study sample. During an acid attack, fluoride will diffuse with the acid and inhibit enamel dissolution (77). If present during remineralization, it enhances crystal growth and encourages mineral precipitation. It may in fact render the enamel more resistant to subsequent attacks (45). Therefore, presence of these elements in our present study suggest there was an increased resistance against demineralization in the study group were light curable fluoride varnish was applied when compared to that of the control group.

EDAX analysis revealed that there was a significant difference in sodium, aluminum, phosphorus, calcium, fluoride, magnesium and iron when compared between the study group and the control group. Whereas oxygen, chlorine silicon and Calcium -Phosphorus ratio is statistically insignificant. These were shown in Table I, II, III, IV.

A statistically significant increase in the weight percentage of sodium, aluminum, fluoride, magnesium and iron were noted in the study group when compared to that of the control group. This increase in aluminum and fluoride can be indicative of the varnish which contain aluminum fluorosilicate glass. The difference in weight percentage of elements oxygen, chlorine, silicon and Calcium -Phosphorus ratio was statistically insignificant. (Table I, II, III, IV).

This study also showed that a significant increase in weight percentage of fluoride and, aluminum, which is the content of the light curable fluoride varnish (Clinpro-XT). In other words, a decrease in phosphorus is dependent on the increase in calcium and fluoride. These findings corroborate with those of others [(35).]

The results of the present study show that one can in fact decrease the formation of very early demineralization of enamel surrounding the orthodontic appliances with the use of fluoride releasing varnish independent of patient cooperation. Sustained release of a low level of fluoride leads to the formation of a calcium-fluoride coating on the enamel surface. This reservoir of fluoride on the enamel surface can provide fluoride for remineralization and calcium for neutralization of the acid attack.

LIMITATIONS

One of the limitation of this study is that the sample size for this study was small due to delayed premolar extraction. Further studies should be carried out with an increased sample size to increase the power of the study.

A second limitation was the duration of the study. The present study samples were obtained for analysis at 60 and 90 days. This time period could not explain the need for repeated application of fluoride in the given time frame.

Summary and Conclusion

SUMMARY AND CONCLUSION

Our study evaluated the effects of light curable fluoride varnish in patients undergoing orthodontic treatment.

SEM micrographs not only allowed for the ultra-structural changes within the enamel but also detected the relative the size of newly formed crystals in the fluoride exposed groups. SEM micrographs revealed roughened enamel surface with multiple areas of enamel erosion in the non-fluoride exposed group which may indicate the presence of acids were noted in both 60 and 90 days. Whereas in the fluoride exposed group around the bracket base irregular distribution of globules suggestive of calcium fluoride like material were observed.

The EDAX analysis revealed a significant amount of increase in the weight percentage of sodium aluminum fluoride magnesium and iron were noted when compared between the fluoride exposed group and the non-fluoride exposed group

Based on the results of the present study it can be concluded that a single application of light curable fluoride varnish (Clinpro -XT) may be used to prevent demineralization by forming a mechanical barrier as well as by slow release of fluoride in a long term clinical situation up to 90 days. It is advisable to use in patients who are non -compliant in using other methods of fluoride intake, such as mouth rinses, gels etc. The time and exposure played a significant role.

Further studies with increased sample sizes and long term clinical situations is warranted to evaluate the effectiveness of a single application of light curable fluoride varnish on enamel demineralization are required to increase the power of the study.

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Annexures

ANNEXURE I

RAGAS DENTAL COLLEGE & HOSPITAL

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TO WHOMSOEVER IT MAY CONCERN

Date: 9/1/2017

From

The Institutional Ethics Board,

Ragas Dental College and Hospital,

Uthandi,

Chennai- 600119

The dissertation topic titled **"TO EVALUATE THE EFFECT OF LIGHT CURABLE FLUORIDE VARNISH ON ENAMEL DEMINERALIZATION DURING ORTHODONTIC TREATMENT-AN IN VIVO STUDY"** submitted by **Dr.Vidhu.S.** has been approved by the Institutional Ethics Board of Ragas Dental College and Hospital.

Dr. N.S. Azhagarasan, MDS.,

Member secretary, Institutional Ethics Board,

Head of the Institution,

Ragas Dental College and Hospital,

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ANNEXURE- II

DECLARATION OF PLAGIARISM CHECK

From,

VIDHU.S
III Post Graduate Student
Department of Orthodontics and Dentofacial Orthopedics
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To,

The Head of the Department
Department of Orthodontics and Dentofacial Orthopedics
Ragas Dental College and Hospital
Chennai.

SUB: Declaration of plagiarism check of my dissertation to be submitted to the "The TamilNadu Dr.M.G.R. Medical University"-April 2017.

I hereby declared that I have checked my dissertation for plagiarism using Small SEO Tools -pliagarism checker software on 9-1-2017 date for this dissertation. The unique content was 92% and the plagiarism content was 8%.The plagiarism content corresponds to definitions and terminologies that have to be quoted.

Vidhu